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PATENT

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In Re Application of: Chapman and King

Confirmation No. 3379

Serial No.: 09/719,045

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Filing Date: December 7, 2000

Examiner: David A. Saunders

For: DIVALENT ANTIBODY FRAGMENTS

Customer No.: 34133

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DATE OF DEPOSIT: June 13, 2006

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

TRANSMITTAL LETTER FOR CERTIFIED COPY OF PRIORITY DOCUMENT

Attached please find the certified copy of the foreign application from which priority is claimed for this case:


Country: Great Britain

Application No.: GB 9812545.3

Filing Date: 10 June 1998

Applicant respectfully requests that receipt of the enclosed document be acknowledged and that the U.S. Patent Office's records are updated accordingly.

Respectfully submitted,


Doreen Yatko Trujillo
Registration No. 35,719

Dated: June 13, 2006

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I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

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The Patent Office

Cardiff Road
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1. Your reference

PA435

2. Patent application number

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9812545.3

110 JUN 1998

3. Full name, address and postcode of the or of each applicant (underline all surnames)

CELLTECH THERAPEUTICS LIMITED
216 BATH ROAD
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SL1 4EN

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

UK
6672877001

4. Title of the invention

BIOLOGICAL PRODUCTS

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

~~PATENT DEPARTMENT~~
~~CELLTECH THERAPEUTICS LIMITED~~
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WC1A 2RA.

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6672877002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
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Date of filing
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
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a) any applicant named in part 3 is not an inventor, or

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11.

I/We request the grant of a patent on the basis of this application.

For and on behalf of CELLTEC THERAPEUTICS LIMITED
Signature Date

Ansell

10th June 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

DR P ANSELL 01753 534655

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BIOLOGICAL PRODUCTS

5 This invention relates to modified divalent antibody fragments, to processes for their preparation, to compositions containing them and to their use in medicine.

10 Antibodies are increasingly being used in the clinic for diagnostic and therapeutic purposes. The aim in each case is to exploit the combination of high specificity and affinity of the antibody-antigen interaction, to enable detection and/or treatment of a particular lesion. The antibody is used alone, or is loaded with another atom or molecule such as a radioisotope or cytotoxic drug.

15 The pharmacokinetics and biodistribution of an antibody play a major role in determining whether its use in the clinic will be successful. Thus the antibody must be capable of being delivered to the site of action and be retained there for a length of time suitable to achieve its purpose. It also should be present only at sub-toxic levels outside of the target and it must be catabolised in a well-defined manner.

20 For many uses the pharmacokinetics of antibodies are not ideal. This is especially true for tumour diagnosis and therapy with antibody-radioisotope or drug conjugates. For diagnosis with such conjugates long half-lives limit the tumour-to-background ratio and hence the sensitivity of lesion detection. For therapy, a long half-life leads to long-term exposure of normal tissues to the antibody conjugate and hence to dose-limiting toxicity.

30 A number of approaches are available to manipulate the pharmacokinetics of antibodies, and these usually also affect their biodistribution. The simplest and most generally applicable approach is the use of antibody fragments. These are cleared more rapidly from the circulation than whole antibodies and distribute more rapidly from the blood to the tissues, which is a particular advantage in some applications, for example for tumour imaging and therapy.

35

- In order to improve the pharmacokinetics of antibody fragments still further we have investigated the use of polymers. The attachment of polymeric materials such as polyethylene glycol (PEG), to protein molecules is well established and it has been demonstrated that attachment of a polymer
- 5 can substantially alter the pharmacological properties of a protein molecule. For example, PEG modification of proteins can alter the *in vivo* circulating half-life of the protein, antigenicity and immunogenicity, solubility, mechanical stability and resistance to proteolysis [Abuchowski, A. *et al* J. Biol. Chem (1977) 252, 3578-3581 and 3582-3586; Nucci, M.
- 10 L. *et al*, Adv. Drug Delivery Reviews (1991) 6, 133-151; Francis, G. *et al*, Pharmaceutical Biotechnology Vol. 3. (Borchardt, R. T. ed.); and Stability of Protein Pharmaceuticals: *in vivo* Pathways of Degradation and Strategies for Protein Stabilization (1991) pp 235-263 (Ahern, T. J and Manning, M., ed.s) Plenum, New York].
- 15 Attachment of PEG to protein molecules has been achieved using a number of different chemical methods, most of which attach PEG to lysine residues or other amino acid residues on the surface of the protein in a random fashion [Zalipsky, S. & Lee, C. Poly(ethylene glycol) Chemistry:
- 20 Biotechnical and Biomedical Applications (1992) pp 347-370 (Harris, J. M., ed), Plenum, New York]. This often leads to partial impairment of the function of the protein, for example enzymes have reduced catalytic activity [Nucci, M. L. *et al* *ibid*].
- 25 Site-specific modification of proteins to introduce sites for PEG attachment has been reported. Interleukin-2, for example, has been modified by mutagenesis to replace a threonine residue which is normally glycosylated by a cysteine to allow attachment of PEG, [Goodson, R. J. & Katre, N. V. Bio/Technology (1990) 8, 343-346]. A site which is normally glycosylated
- 30 was chosen as this was thought to be capable of tolerating PEG modification without perturbation of the protein structure. In another example, the enzyme purine nucleoside phosphorylase has been modified to selectively replace arginine residues with lysines to provide in this instance up to eighteen additional potential PEG attachment sites per
- 35 enzyme molecule [Hershfield, M. S. *et al* P.N.A.S. (1991), 88, 7185-7189].

Previous studies with antibodies and antibody fragments have used random PEG attachment via lysine residues [e.g. Ling, T. G. I. & Mattiasson, B. J. Immunol. Methods (1983), 59, 327-337; Wilkinson, I. *et al* Immunol. Letters (1987) 15, 17-22; Kitamura, K. *et al* Cancer Res. (1991), 51, 4310-4315; Delgado, C. *et al* Br. J. Cancer (1996), 73, 175-182] and thiolated derivatives [Pedley, R. B. *et al* Br. J. Cancer (1994), 70, 1126-1130]. Random attachment has often resulted in modified antibodies which are only able to bind their target antigen with reduced affinity, avidity or specificity. In one attempt to overcome this, critical lysine residues in antigen binding (CDR) loops have been replaced with arginines to allow modification with less loss in immunoreactivity [Benhar, I. *et al* Bioconjugate Chemistry (1994) 5, 321-326].

Specific sites in the constant and the hinge regions of antibodies can be engineered to allow site-specific linkage of a range of effector and reporter molecules [Lyons, A. *et al* Prot. Eng. (1990), 3, 703-709; and European Patent Specifications Nos. 348442 and 347433]. We have now determined that site-specific attachment of polymers to divalent antibody fragments can be used to avoid the loss of immunoreactivity previously associated with random attachment processes. Furthermore, fragments modified in this way have markedly improved binding and/or pharmacokinetic properties when compared to fragments which have been modified randomly with the same number and type of polymer molecules.

Thus according to one aspect of the invention we provide a divalent antibody fragment comprising two antibody heavy chains and at least one polymer molecule in covalent linkage, each heavy chain being covalently linked to the other by at least one non-disulphide interchain bridge linking the sulphur atom of a cysteine residue in one chain to the sulphur atom of a cysteine residue in the other chain, said cysteine residues being located outside of the variable region domain of each chain, characterised in that at least one non-disulphide interchain bridge contains a covalently linked polymer molecule.

The term "non-disulphide" as used herein is intended to mean that S-S bridges, e.g. of the type normally found in antibodies, are excluded. An

interchain bridge of the type present in a fragment according to the invention may however still be linked to a heavy chain via a -S-S- bond as described hereinafter.

- 5 The antibody fragment of the invention will in general be capable of selectively binding to an antigen. The antigen may be any cell-associated antigen, for example a cell surface antigen such as a T-cell, endothelial cell or tumour cell marker, or it may be a soluble antigen. Particular examples of cell surface antigens include adhesion molecules, for
10 example integrins such as $\beta 1$ integrins, e.g. VLA-4, E-selectin, P-selectin or L-selectin, CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD20, CD23, CD25, CD33, CD38, CD40, CD45, CDW52, CD69, carcinoembryonic antigen (CEA), human milk fat globulin (HMFG1 and 2), MHC Class I and MHC Class II antigens, and VEGF, and where
15 appropriate, receptors thereof. Soluble antigens include interleukins such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 or IL-12, viral antigens, for example respiratory syncytial virus or cytomegalovirus antigens, immunoglobulins, such as IgE, interferons such as interferon- α , interferon- β or interferon- γ , tumour necrosis factor- α , tumour necrosis factor- β , colony stimulating
20 factors such as G-CSF or GM-CSF, and platelet derived growth factors such as PDGF- α , and PDGF- β and where appropriate receptors thereof.

In order to achieve useful antigen binding properties each heavy chain in a fragment according to the invention may be paired with a complementary
25 antibody light chain or a fragment thereof and the invention extends to such constructs. Where desired, a heavy-light chain pair may be in covalent linkage, for example a disulphide linkage as found in naturally occurring antibodies and/or a peptide linkage as found for example in recombinant single chain antibodies.

30 In general each heavy chain and, when present, light chain, will have a variable region domain. The term variable region domain as used herein is intended to mean that part of a heavy or light chain which contains the antigen binding site (hereinafter a V_H or V_L domain). The V_H or V_L
35 domain may be of any size or amino acid composition and will generally

comprise at least one hypervariable amino acid sequence responsible for antigen binding embedded in a framework sequence.

- Each V_H or V_L domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain which has been created using recombinant DNA engineering techniques. Such engineered versions include those created for example from natural antibody variable regions by insertions, deletions or changes in or to the amino acid sequences of the natural antibodies.
- 5 Particular examples of this type include those engineered V_H or V_L domains containing at least one CDR and optionally one or more framework amino acids from one antibody and the remainder of the variable region domain from a second antibody.
- 10 Each V_H domain will generally be covalently attached to at least one cysteine residue. The location of each cysteine residue may be varied according to the size and nature of the antibody fragment required. Thus, in one extreme example a cysteine residue may be attached directly to the C-terminal amino acid of the V_H domain. This may then function as the
- 15 bridging site for an interchain bridge containing a polymer molecule. Two V_H domains of this type may thus be bridged to form a fragment according to the invention.
- 20

- In practice however, it is generally preferable that the V_H domain is covalently attached at the C-terminal amino acid to at least one other antibody domain or a fragment thereof which contains a cysteine residue. Thus, for example a V_H domain may be linked to an immunoglobulin C_H1 domain or a fragment thereof. The C_H1 domain may be extended with further amino acids, for example to provide a hinge region domain as
- 25 found generally in immunoglobulins, or to provide further domains, such as antibody $CH2$ and $CH3$ domains. In each of the above cases at least one cysteine residue may be located at any point throughout any domain to form a bridging site for an interchain bridge containing a polymer molecule.
- 30

Similarly any V_L domain present in a fragment according to the invention may be attached to an antibody light chain constant domain (C_L) or a fragment thereof.

5 The polymer molecule in the fragment according to the invention may in general be a synthetic or naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or heteropolysaccharide.

10

Particular optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups. Particular examples of synthetic polymers include optionally substituted straight or branched chain poly(ethylene glycol),
15 poly(propylene glycol), or poly(vinyl alcohol) and derivatives thereof, especially optionally substituted poly(ethylene glycol) such as methoxy(polyethylene glycol) and derivatives thereof. Particular naturally occurring polymers include lactose, amylose, dextran or glycogen and derivatives thereof. "Derivatives" as used herein is intended to include
20 reactive derivatives, for example active esters such as succinimidyl esters and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product of the invention as the linking group between the polymer and the interchain bridge.

25

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from around 500Da to around 50000Da for example from 5000 to 40000Da and including 25000 to 40000Da. The polymer size may in particular be selected on the basis of
30 the intended use of the product. Thus for example where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example around 5000Da. For applications where the product remains in the circulation it may be advantageous to
35 use a higher molecular weight polymer, for example in the range 25000Da to 40000Da.

In general, each polymer molecule in the antibody fragment according to the invention forms part of an interchain bridge. Each bridge serves to link two heavy chains and in each chain will be covalently linked to a sulphur atom of a cysteine residue. The covalent linkage will generally be a disulphide bond or, in particular a sulphur-carbon bond.

Each interchain bridge may in general be of any desired length or composition. Suitable bridges include residues of homo- or heterofunctional cross-linking reagents, particularly homo- or heterobifunctional cross-linking reagents containing one or more covalently linked polymer molecules as just described.

Homo- or heterofunctional cross-linking reagents include polyvalent, especially bivalent radicals of aliphatic, heteroaliphatic, cycloaliphatic, heterocycloaliphatic, aromatic or heteroaromatic groups containing two thiol reactive functional groups. Each fragment according to the invention will have an interchain bridge derived from such a reagent in which each thiol reactive functional group is in covalent linkage with a sulphur atom of a cysteine residue. Particular thiol reactive functional groups include α -halocarboxylic acids or esters, e.g. iodoacetamide, imides, e.g. maleimide, vinyl sulphones or disulphides.

Particular bridges include optionally substituted straight or branched C₄₋₂₀ alkylene, C₄₋₂₀alkenylene or C₄₋₂₀alkynylene chains optionally interrupted by one or more heteroatoms or heteroatom-containing groups such as -O- or -S- atoms or -N(R¹)- [where R¹ is a hydrogen atom or a C₁₋₆alkyl group], -CON(R¹)-, -N(R¹)CO-, -SO₂N(R¹)-, -N(R¹)SO₂-, -C(O)-, -S(O)-, -S(O)₂-, -OCON(R¹)-, -N(R¹)C(O)O-, -C(O)O- groups, or by cyclopentylene, cyclohexylene, phenylene or substituted phenylene groups. Optional substituents include for example one or more amino or substituted amino groups, e.g. -N(R¹)₂ groups where each R¹ atom or group may be the same or different.

The polymer may be covalently attached at any location in the interchain bridge, generally through a heteroatom or heteroatom-containing group as just described in relation to particular interchain bridges.

- 5 Particularly useful fragments according to the invention are those containing a single interchain bridge. In these particular fragments the polymer may especially be a synthetic polymer, particularly a polyalkylene polymer such as poly(ethylene glycol) or especially methoxypoly(ethylene glycol) or a derivative thereof, and especially with a molecular weight in
- 10 the range from about 25000Da to about 40000Da. The bridge may in particular be an optionally substituted straight or branched C₄₋₂₀alkylene chain optionally interrupted by one or more heteroatoms or heteroatom-containing groups as described previously.
- 15 Each heavy chain in the fragments according to the invention is preferably a V_H-CH1 chain terminally substituted by a hinge region domain, for example as naturally found in immunoglobulins. Each chain is preferably paired with a light chain, particularly a V_H-C_L chain, thus for example forming a Fab fragment. In preferred fragments of the invention containing
- 20 heavy or heavy-light chain pairs of these types a single interchain bridge will be present, particularly bridging a cysteine residue located in the hinge sequence of each heavy chain. Desirably this will be the only cysteine residue present in each hinge sequence.
- 25 Where desired, the antibody fragment according to the invention may additionally have one or more effector or reporter molecules attached to it and the invention extends to such modified antibodies. The effector or reporter molecules may be attached to the antibody fragment through any available amino acid side-chain or terminal amino acid functional group
- 30 located in the fragment, for example any free amino, imino, hydroxyl or carboxyl group.

Effector molecules include, for example, antineoplastic agents, toxins (such as enzymatically active toxins of bacterial or plant origin and

35 fragments thereof e.g. ricin and fragments thereof) biologically active proteins, for example enzymes, nucleic acids and fragments thereof, e.g.

DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, and chelated metals. Suitable reporter groups include chelated metals, fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

5

Particular antineoplastic agents include cytotoxic and cytostatic agents, for example alkylating agents, such as nitrogen mustards (e.g. chlorambucil, melphalan, mechlorethamine, cyclophosphamide, or uracil mustard) and derivatives thereof, triethylenephosphoramidate, triethylenethiophosphoramide, busulphan, or cisplatin; antimetabolites, such as methotrexate, fluorouracil, floxuridine, cytarabine, mercaptopurine, thioguanine, fluoroacetic acid or fluorocitric acid, antibiotics, such as bleomycins (e.g. bleomycin sulphate), doxorubicin, daunorubicin, mitomycins (e.g. mitomycin C), actinomycins (e.g. dactinomycin) plicamycin, calichaemicin and derivatives thereof, or esperamicin and derivatives thereof; mitotic inhibitors, such as etoposide, vincristine or vinblastine and derivatives thereof; alkaloids, such as ellipticine; polyols such as taxicin-I or taxicin-II; hormones, such as androgens (e.g. dromostanolone or testolactone), progestins (e.g. megestrol acetate or medroxyprogesterone acetate), estrogens (e.g. dimethylstilbestrol diphosphate, polyestradiol phosphate or estramustine phosphate) or antiestrogens (e.g. tamoxifen); anthraquinones, such as mitoxantrone, ureas, such as hydroxyurea; hydrazines, such as procarbazine; or imidazoles, such as dacarbazine.

25 Particularly useful effector groups are calichaemicin and derivatives thereof (see for example South African Patent Specifications Nos. 85/8794, 88/8127 and 90/2839).

Chelated metals include chelates of di-or tripositive metals having a coordination number from 2 to 8 inclusive. Particular examples of such metals include technetium (Tc), rhenium (Re), cobalt (Co), copper (Cu), gold (Au), silver (Ag), lead (Pb), bismuth (Bi), indium (In), gallium (Ga), yttrium (Y), terbium (Tb), gadolinium (Gd), and scandium (Sc). In general the metal is preferably a radionuclide. Particular radionuclides include
 30 ^{99m}Tc , ^{186}Re , ^{188}Re , ^{58}Co , ^{60}Co , ^{67}Cu , ^{195}Au , ^{199}Au , ^{110}Ag , ^{203}Pb ,
 35 ^{206}Bi , ^{207}Bi , ^{111}In , ^{67}Ga , ^{68}Ga , ^{88}Y , ^{90}Y , ^{160}Tb , ^{153}Gd and ^{47}Sc .

The chelated metal may be for example one of the above types of metal chelated with any suitable polydentate chelating agent, for example acyclic or cyclic polyamines, polyethers, (e.g. crown ethers and derivatives thereof); polyamides; porphyrins; and carbocyclic derivatives.

In general, the type of chelating agent will depend on the metal in use. One particularly useful group of chelating agents in conjugates according to the invention, however, are acyclic and cyclic polyamines, especially polyaminocarboxylic acids, for example diethylenetriaminepentaacetic acid and derivatives thereof, and macrocyclic amines, e.g. cyclic tri-aza and tetra-aza derivatives (for example as described in International Patent Specification No. WO 92/22583); and polyamides, especially desferrioxamine and derivatives thereof.

The antibody fragment according to the invention may be prepared by reacting an antibody fragment containing a heavy chain reactive cysteine residue located outside of the V_H domain with a thiol-selective cross-linking reagent containing a polymer as defined herein. The reaction may generally be performed in a solvent, for example an aqueous buffer solution such as an acetate or phosphate buffer, at around neutral pH, for example around pH 4.5 to around pH 8.0, at for example ambient temperature. The antibody will generally be employed in excess concentration relative to the concentration of the cross linking reagent. In some instances it may be necessary to reduce the antibody starting material with a reagent such as β -mercaptoethylamine (for example as described in Example 1 hereinafter) to generate an appropriately reactive cysteine residue. Where necessary, the desired product may be separated from any unreacted starting materials or any other unwanted product generated during the production process by conventional means, for example by chromatography.

The antibody fragment starting material may be obtained from any whole antibody, especially a whole monoclonal antibody, [prepared by conventional immunisation and cell fusion procedures], using any suitable standard enzymatic cleavage and/or digestion techniques, for example by

treatment with pepsin. Alternatively, the antibody starting material may be prepared by the use of recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and/or constant regions. Such DNA is known and/or is readily available from DNA libraries including for example phage-antibody libraries [see Chiswell, D J and McCafferty, J. *Tibtech.* 10 80-84 (1992)] or where desired can be synthesised. Standard molecular biology and/or chemistry procedures may be used to sequence and manipulate the DNA, for example, to introduce codons to create cysteine residues, to modify, add or delete other amino acids or domains as desired.

From here, one or more replicable expression vectors containing the DNA may be prepared and used to transform an appropriate cell line, e.g. a non-producing myeloma cell line, such as a mouse NSO line or a bacterial, e.g. *E.coli* line, in which production of the antibody fragment will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operably linked to the variable domain sequence. Particular methods for producing antibody fragments in this way are generally well known and routinely used. For example, basic molecular biology procedures are described by Maniatis *et al* [Molecular Cloning, Cold Spring Harbor Laboratory, New York, 1989]; DNA sequencing can be performed as described in Sanger *et al* [PNAS 74, 5463, (1977)] and the Amersham International plc sequencing handbook; and site directed mutagenesis can be carried out according to the method of Kramer *et al* [Nucl. Acids Res. 12, 9441, (1984)] and the Anglian Biotechnology Ltd handbook. Additionally, there are numerous publications, including patent specifications, detailing techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors and transformation of appropriate cells, for example as reviewed by Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews [ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK] and in International Patent Specification No. WO 91/09967.

The thiol-selective cross-linking reagent for use in the preparation of antibody fragments according to the invention may be obtained by reaction

of any thiol-reactive cross-linking agent (containing for example thiol reactive groups such as an α -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone, or a disulphide) with an appropriately functionalised polymer. Suitable polymer starting materials may be obtained commercially (for example from Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures, for example as described by Zalipsky, S & Lee, C, *ibid*. The reaction may in general be a conventional coupling reaction between the functionalised polymer, for example an active ester of the polymer, and an appropriate functional group, for example an amine, present in the cross-linking reagent. Standard reaction conditions may be used, for example as described in the experimental section hereinafter for coupling an active ester with an amine. Suitable cross-linking reagents are readily available from commercially available sources or may be simply synthesised using conventional procedures from commercially available materials, for example as described in European Patent Specification No. 384624 and International Patent Specification No. WO 92/22583 and the experimental section hereinafter.

Where it is desired to obtain an antibody fragment according to the invention linked to an effector or reporter molecule this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector or reporter molecule either before or after reaction with the activated polymer as appropriate. Particular chemical procedures include for example those described in International Patent Specification Nos. WO 93/06231, WO 92/22583, WO 90/09195 and WO 89/01476. Alternatively, where the effector or reporter molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in International Patent Specification No. WO 86/01533 and European Patent Specification No. 392745.

The antibody fragment according to the invention may be useful in the detection or treatment of a number of diseases or disorders. Such diseases or disorders may include those described under the general

headings of infectious disease, e.g. viral infection; inflammatory disease/autoimmunity e.g. rheumatoid arthritis, osteoarthritis, inflammatory bowel disease; cancer; allergic/atopic disease e.g. asthma, eczema; congenital disease, e.g. cystic fibrosis, sickle cell anaemia; dermatologic disease, e.g. psoriasis; neurologic disease, e.g. multiple sclerosis; transplants e.g. organ transplant rejection, graft-versus-host disease; and metabolic/idiopathic disease e.g. diabetes.

The antibody fragments according to the invention may be formulated for use in therapy and/or diagnosis and according to a further aspect of the invention we provide a pharmaceutical composition comprising a modified monovalent antibody fragment comprising a monovalent antibody fragment and at least one polymer molecule in covalent linkage characterised in that each covalent linkage is through a sulphur atom of a cysteine residue located in the antibody fragment outside of the variable region domain of the fragment, together with one or more pharmaceutically acceptable excipients, diluents or carriers.

As explained above, the antibody fragment in this aspect of the invention may be optionally linked to one or more effector or reporter groups.

The pharmaceutical composition may take any suitable form for administration, and, preferably is in a form suitable for parenteral administration e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the composition is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents such as suspending, preservative, stabilising and/or dispersing agents.

Alternatively, the antibody composition may be in dry form, for reconstitution before use with an appropriate sterile liquid.

If the antibody composition is suitable for oral administration the formulation may contain, in addition to the active ingredient, additives such as: starch e.g. potato, maize or wheat starch or cellulose or starch derivatives such as microcrystalline cellulose; silica; various sugars such

as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end, it may be desirable to include in the formulation mucus formers and resins. It may
 5 also be desirable to improve tolerance by formulating the antibody in a capsule which is insoluble in the gastric juices. It may also be preferable to include the antibody or composition in a controlled release formulation.

If the antibody composition is suitable for rectal administration the
 10 formulation may contain a binding and/or lubricating agent; for example polymeric glycols, gelatins, cocoa-butter or other vegetable waxes or fats.

Therapeutic and diagnostic uses of fragments according to the invention typically comprise administering an effective amount of the antibody
 15 fragment to a human subject. The exact amount to be administered will vary according to the use of the antibody and on the age, sex and condition of the patient but may typically be varied from about 0.1mg to 1000mg for example from about 1mg to 500mg. The antibody may be administered as a single dose or in a continuous manner over a period of
 20 time. Doses may be repeated as appropriate. Typical doses may be for example between 0.1-50mg/kg body weight per single therapeutic dose, particularly between 0.1-20 mg/kg body weight for a single therapeutic dose.

25 The following Examples illustrate the invention.

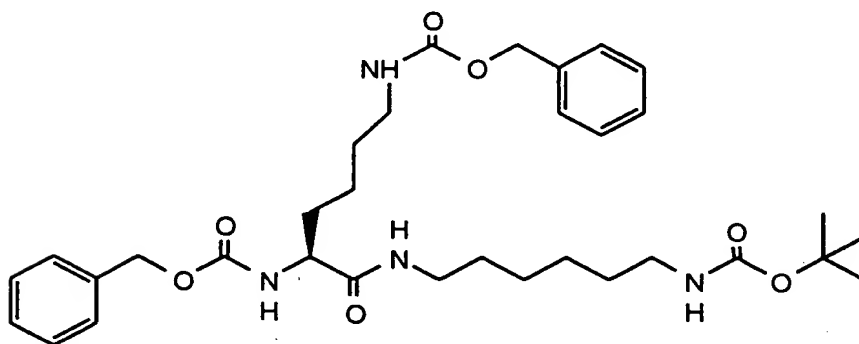
The following abbreviations are used:

PEG	-	$\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2-$
DFM-PEG	-	an antibody fragment according to the invention in which two Fab' fragments are cross-linked with a PEGylated dimaleimide bridge.
30 DTDP	-	4,4'-dithiodipyridine
AUC	-	area under the curve
RT	-	room temperature
TFA	-	trifluoroacetic acid
35 BOC	-	<i>tert</i> butoxycarbonyl
CBZ	-	carbobenzyloxy

DMF	-	N,N-dimethylformamide
EDC	-	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
BMH	-	bismaleimido-hexane

5 Preparation of Intermediate Bridging Groups

INTERMEDIATE 1

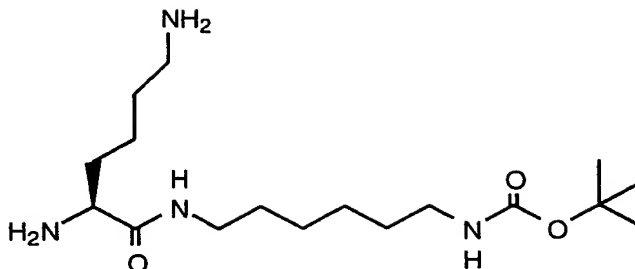


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N(α)N(ϵ)Di-CBZ-(L)-Lysine (18.0g, 43.3mmol) was dissolved in anhydrous DMF (80ml). N-BOC-1,6-diaminohexane hydrochloride (11.06, 43.75mmol), hydroxybenzotriazole hydrate (6.43g, 47.63mmol), 4-methylmorpholine (5.2ml, 47.63mmol) and EDC (9.13g, 47.63mmol) were added and the mixture stirred at room temperature for 5h. The reaction mixture was poured into H₂O and extracted with ethyl acetate (4 x 100ml). The combined organic layers were washed with 10% citric acid (2 x 50ml), saturated NaHCO₃ (2 x 50ml), brine (1 x 50ml) and dried over MgSO₄. Removal of the solvent *in vacuo* gave Intermediate 1 (26.15g, 100%) as a white solid.

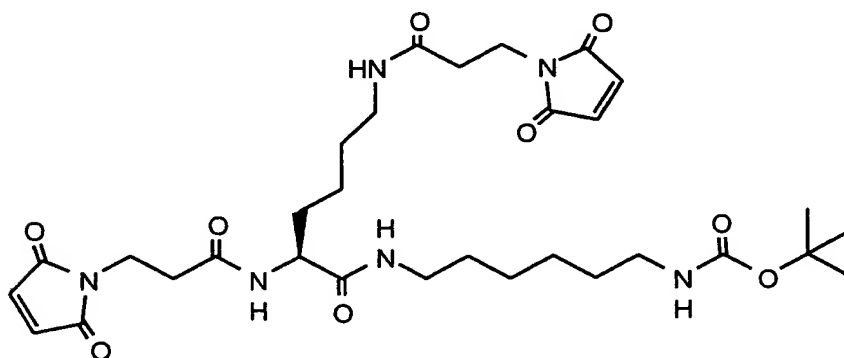
20

¹H NMR ((CD₃)₂SO) δ 7.79 (1H, t), 7.38-7.19 (11H, m), 6.72 (1H, t), 5.00-4.99 (4H, m), 3.95-3.90 (1H, m), 3.08-2.87 (6H, m) and 1.60-1.05 (23H, m including 9H, s).

INTERMEDIATE 2

5

Intermediate 1 (26.15g, 42.7mmol) was dissolved (with warming) in ethanol (700ml) and treated with 10% Pd/C (4.2g). The reaction was stirred under H₂ gas in a hot water bath (~30°) for 4h and then allowed to cool. CH₂Cl₂ (20ml) was added and the mixture was filtered and washed well with CH₂Cl₂ and ethanol. Removal of the solvent *in vacuo* gave Intermediate 2 as an oily foam (13.8g, 94%). ¹H NMR ((CH₃)₂SO) δ 7.78 (1H, t), 3.06-2.91 (3H, m), 2.89-2.84 (4H, m), 2.52-2.43 (4H, bs) and 1.57-1.15 (23H, m. including 9H, s).

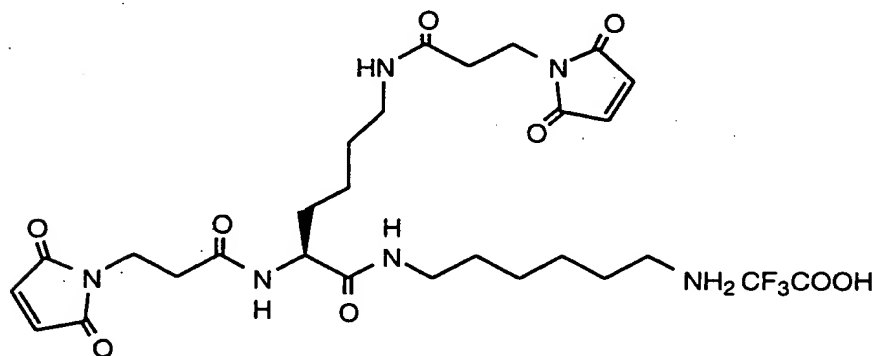
15 **INTERMEDIATE 3**

Intermediate 2 (1.47g, 4.47mmol) was dissolved in anhydrous DMF (25ml) and N-succinimidyl 3-maleimidopropionate (22.38g, 8.97mmol) added. The reaction mixture was stirred at room temperature for 3h. The solvent was removed *in vacuo*, and CH₂Cl₂ (50ml) was added followed by saturated NaHCO₃ (50ml). The layers were separated and the aqueous

20

5

INTERMEDIATE 4



20

25

Preparation of PEGylated (40KDa) bridging group

To Intermediate 4 in DMF was added one molar equivalent of N-methyl morpholine and a five-fold molar excess of amine-reactive PEG (40kDa

PEG-NHS ester; Shearwater Polymers Inc. Huntsville, AL, USA). The mixture was incubated at RT for 2 hours with occasional agitation. Unreacted PEG was quenched with a 100-fold molar excess of glycine over PEG, added from a stock solution of 1M glycine in 0.1M phosphate buffer, pH6.0 containing 2mM EDTA, and the mixture incubated for a minimum of a further 10 minutes to obtain the desired PEGylated bridging group.

Preparation of Fab'

10 Fab' from the engineered human antibody g162, which recognises PDGF β receptor, was expressed in E. coli as described in International Patent Application No. PCT/GB97/03400. The Fab' fragment has a single cysteine residue present in its hinge region available for cross-linking. Cells were harvested from fermentation culture by centrifugation and Fab' 15 extracted by resuspending cells in 100mM tris pH7.4 containing 10mM EDTA and incubating at 60°C overnight. Fab' was then purified by expanded bed chromatography using a column of Streamline ATM (Pharmacia) which was pre-equilibrated with 1M glycine/glycinate pH8.0. The sample was made 1M with respect to glycine and the pH adjusted 20 to 7.5 with 50% (w/v) sodium glycinate before application to the column in expanded bed mode. After washing with equilibration buffer, the column material was packed into a packed bed and Fab' was eluted with 0.1M citrate pH3.0.

25 Further purification was achieved by adjusting the pH of the eluate to 7.5 with 2M tris and applying to a column of Protein G sepharose pre-equilibrated with phosphate buffered saline pH7.4. After washing with equilibration buffer, Fab' was eluted with 0.1M glycine-HCl pH2.7. The pH of the eluted Fab' was then adjusted to 6.0 with 2M tris.

30

Preparation of anti-PDGF β R DFM-PEG (site-specific)

Purified anti-PDGF β R Fab' was diafiltered into 0.1M phosphate buffer, pH6.0 containing 2mM EDTA. The hinge thiol was activated by reduction with β -mercaptoethylamine. Fab' was incubated with 5mM β -mercaptoethylamine in 0.1M phosphate buffer, pH6.0 containing 2mM 35 EDTA for 30 minutes at 37°. The sample was then desalted into 0.1M

phosphate buffer, pH6.0 containing 2mM EDTA, using Sephadex G-25 (PD10) columns. The number of thiol groups per Fab' molecule was measured by titration with DTDP as previously described [Lyons *et al* (1990), *ibid*]. The Fab' was cross-linked with the PEGylated cross-linker prepared from Intermediate 4 at a Fab':linker molar ratio of 2.2:1, at 37°. The cross-linker was added in 5 aliquots at 5 minute intervals, and the sample incubated for >1h.

The desired DFM-PEG was purified by gel filtration chromatography using Sephacryl S-400 HR (to remove unreacted Fab') in 50mM acetate buffer pH4.5, followed by cation exchange chromatography using Mono S to separate DFM-PEG from DFM and Fab'-PEG. Mono S chromatography was carried out using a column equilibrated with 50mM acetate pH 4.5, after application of sample and washing with equilibration buffer, bound material was eluted using a linear gradient of sodium chloride. Purified material was examined on SDS-PAGE and shown to have a slower mobility than unmodified DFM or Fab' demonstrating successful conjugation of PEG (Figure 1).

20 **Preparation of randomly PEGylated anti-PDGFR DFM-PEG**

For comparative purposes anti-PDGFR DFM was prepared and derivatised with PEG randomly. Anti-PDGFR Fab' was reduced as described above. The Fab' was cross-linked with BMH dissolved in DMF at a Fab':BMH molar ratio of 2.2:1, at 37°. The cross-linker was added in 5 aliquots at 5 minute intervals, and the sample incubated for >1h. The resulting DFM (diFab' cross-linked with BMH) was purified from unreacted Fab' by hydrophobic interaction chromatography using phenyl Sepharose HP.

30 The purified DFM was buffer-exchanged into 0.1M phosphate pH8.0 containing 2mM EDTA. Thiols were introduced randomly onto lysine residues by reaction with a 4-fold molar excess of 2-iminothiolane (Traut's reagent) for 1h at RT. After desalting into 0.1M phosphate pH6.0 containing 2mM EDTA using a PD10 column, the number of thiol groups introduced was determined using titration with DTDP. The thiolated DFM was then reacted with a 3.5-fold molar excess of PEG-maleimide

(Shearwater Polymers Inc, *ibid*) over thiols for three hours. An average of 1.3 PEG molecules were attached per DFM as quantified by gel-filtration HPLC analysis. The PEG-DFM was purified by cation exchange chromatography using Mono S. Mono S chromatography was carried out using a column equilibrated with 50mM acetate pH 4.5, after application of sample and washing with equilibration buffer, bound material was eluted using a linear gradient of sodium chloride.

Antigen binding analysis by BiAcure

Kinetic analysis to determine the on and off rates for anti-PDGFβR DFM-PEG binding to PDGFβR was performed using a BIACORE 2000 (Biacore AB). The assay involves capture of a mIgG Fc-PDGFβR fusion molecule by an anti-mouse IgG, which is immobilised on the sensor chip surface, followed by an injection of anti- PDGFβR DFM-PEG. Affinipure F(ab')₂ fragment of goat anti-mouse Ig, Fc fragment specific (Jackson ImmunoResearch) was immobilised on a Sensor Chip CM5 via amine coupling chemistry to a level of 11500RU. A blank surface was prepared by following the immobilisation procedure but omitting injection of the capturing molecule. HBS buffer (10mM HEPES pH7.4, 0.15M NaCl, 3mM EDTA, 0.005% Surfactant P20, Biacore AB) was used as the running buffer with a flow rate of 10ml/min. An injection of mIgG Fc-PDGFβR from COS cell supernatant was captured by the immobilised anti-mouse IgG to a level between 200-250RU. Anti- PDGFβR DFM-PEG molecules were titrated over the captured mIgG Fc- PDGFβR surface from 2mg/ml to 0.52mg/ml. Surfaces were regenerated by injecting 10ml of 30mM hydrochloric acid. Injections of mIgG Fc- PDGFβ R and each concentration of anti- PDGFβR DFM-PEG were repeated over the blank surface as controls. The sensorgram for each anti- PDGFβR DFM-PEG concentration was corrected with the corresponding sensorgram for the blank surface after deletion of the mIgG Fc- PDGFβR injection and regeneration step. Kinetic parameters were calculated using BIAevaluation 2.1 software.

Results for anti-PDGFβR DFM-PEG, both prepared from PEGylated Intermediate 4 (site-specific) and randomly derivatised anti-PDGFβR DFM-PEG are shown in Table 1. Unmodified DFM prepared with BMH as

cross-linker and IgG were used to compare binding parameters. Binding affinity as quantified by the K_d value was similar between the IgG and unmodified DFM at 1.07×10^{-10} M and 1.27×10^{-10} M respectively. Random modification with PEG (1.3 per di-Fab') resulted in a substantial loss of binding affinity to 8.97×10^{-10} M. Site-specific PEGylation with the same size PEG molecule resulted in much improved binding affinity with a K_d value of 2.10×10^{-10} M.

Table 1: BIAcore analysis of DFM-PEG 40kDa compared to IgG and unmodified DFM.

Sample	k _{ass}	k _{diss}	K _d (M)
IgG	9.74×10^6	1.04×10^{-4}	1.07×10^{-10}
DFM	1.81×10^7	2.29×10^{-3}	1.27×10^{-10}
DFM-PEG 40kDa (random)	4.65×10^6	4.17×10^{-3}	8.97×10^{-10}
DFM-PEG 40kDa (site-specific)	8.90×10^6	1.88×10^{-3}	2.10×10^{-10}

Pharmacokinetics

For pharmacokinetic analysis, samples were radiolabelled with ^{125}I using Bolton-Hunter reagent by standard methodology and desalted into phosphate buffered saline at pH 6.8 to remove unreacted ^{125}I . Groups of six male wistar rats were injected i.v. into the tail vein with 20mg of labelled material. At selected time points, blood samples were taken, counted in a gamma counter, and the percent injected dose per gram of blood calculated. The clearance rates and area under the curve values were determined using the SIPHAR software package.

Results (Figure 2) demonstrated slower blood clearance for DFM-PEG conjugate compared to unmodified DFM. This was also reflected in the calculation of pharmacokinetic parameters. Pharmacokinetics of IgG and DFM were best fitted to a two compartment model, whereas DFM-PEG was best fitted to a single compartment model. Results demonstrated a

significantly longer half-life and increased area-under-the-curve for DFM-PEG compared to DFM (Table 2).

Table 2 Pharmacokinetic parameters of anti-PDGF β R IgG, DFM and DFM-PEG (40kDa) site-specific.

	t1/2 α (hours)	t1/2 β (hours)	AUC (0-) (%dose x h)	AUC (% of IgG value)
IgG	5.3 +/- 1.3	95.9 +/- 10.9	6442 +/- 525	100
DFM	0.86 +/- 0.1	28.7 +/- 11.6	283 +/- 71	4.4
DFM-PEG (40kDa)	-----	33.6 +/- 4.5	5318 +/- 1190	82

Bioassay

The potency of anti-PDGF β R DFM and DFM-PEG samples was tested by their ability to block 3H thymidine incorporation by SK-5 dermal fibroblasts in response to PDGF BB. SK-5 cells (grown in DMEM + 10% heat inactivated fetal calf serum, 1% glutamine, 1% sodium pyruvate and 0.025 M HEPES buffer) were trypsinised at 80% confluence and seeded at 5,000 cells/0.1 ml per well in a 96 well tissue culture plate, in serum-free media (1:1 DMEM: HAM's F12 + 5 ug/ml insulin, 16 ng/ml selenium, 20 ug/ml transferrin, 1 mg/ml bovine serum albumin, 1% glutamine, 0.025 M HEPES buffer and pen. strep.) Cells were placed in a 37°, 5% CO₂, 95% humidity, incubator for 24h to quiesce. Media alone, antibody alone, PDGF BB at 10 ng/ml or 20 ng/ml final concentration, or PDGF BB together with varying concentrations of antibody were added to the wells, to a final volume of 0.2 ml. Between 5 and 10 wells were used for each condition. 6-8h later, 3H thymidine (0.5 mCi per well) was added and cells left overnight. Plates were removed from the incubator and placed at -20° for 24h in order to facilitate harvesting. Plates were thawed and DNA harvested onto filter mats using a Skatron Micro96 Harvester. Mats were dried at 67° for 90 min, Betaplate Scint (Wallac) added and mats counted in a LKB Wallac 1205 BETAPLATER liquid scintillation counter.

At 10 ng/ml PDGF BB, and 10 mg/ml of anti-PDGF β R DFM or DFM-PEG, incorporation of ³H thymidine is inhibited by 85-92% by all forms of the antibody. As the concentration of antibody is decreased, the difference between random PEG attachment and site-specific attachment becomes clear (Figure 3). This is equally shown by increasing the concentration of PDGF BB to 20 ng/ml (Figure 4). There is at least a two-fold decrease in potency between the randomly PEG modified 40kDa DFM and the site-specific 40kDa DFM.

10 **EXAMPLE 2**

Site-specific attachment of 10kDa and 20kDa PEG succinimidyl succinate derivatives

Intermediate 4 was derivatised with 20kDa PEG-succinimidyl succinate (Polymer Labs), or 10kDa PEG-succinimidyl succinate (Polymer Labs), and used to prepare anti-PDGF β R DFM-PEG as described in Example 1. In this case purification of the DFM-PEG conjugates was achieved using ion-exchange chromatography on Mono-S. Mono S chromatography was carried out using a column equilibrated with 50mM acetate pH 4.5, after application of sample and washing with equilibration buffer, bound material was eluted using a linear gradient of sodium chloride. Following this step DFM-PEG was further purified using gel filtration on Sephacryl S-200 run in phosphate buffered saline diluted 1:1 with water. SDS-PAGE analysis revealed successful attachment of PEG had taken place (Figure 1).

25 Analysis of antigen binding was carried out by BIAcore analysis as described in Example 1. Results shown in Table 3 demonstrate that these PEG derivatives can be attached with little loss in antigen binding affinity.

Table 3 BIAcore analysis of DFM-PEG prepared with succinimidyl succinate (SS) linkage.

Sample	k _{ass}	k _{diss}	K _d (M)
DFM	1.81×10^7	2.29×10^{-3}	1.27×10^{-10}
DFM-PEG 10kDa (SS linkage)	1.53×10^7	2.18×10^{-3}	1.42×10^{-10}
DFM-PEG 20kDa (SS linkage)	1.20×10^6	2.06×10^{-3}	1.73×10^{-10}

5

These DFM-PEG conjugates were then examined in a pharmacokinetic study in rats using the method described in Example 1. Results (Figure 5) demonstrate significantly slower blood clearance, (longer in vivo half-life) for the DFM-PEG conjugates compared to unmodified DFM.

10

EXAMPLE 3

Preparation of DFM-PEG conjugates using 5KDa PEG-SCM, 20KDa PEG-SPA, 10K PEG2-NHS and 20K PEG2-NHS derivatives.

Intermediate 4 was derivatised with 20kDa PEG-succinimidyl propionate (Shearwater Polymers Inc.*ibid*), or 5kDa PEG-succinimidyl ester of carboxy-methylated PEG (Shearwater Polymers Inc.), or 10KDa PEG2-succinimide (2 x 5KDa, Polymer Labs) or 20KDa PEG2-succinimide (2 x 10KDa, Polymer Labs), and used to prepare anti-PDGFR DFM-PEG conjugates as described in Examples 1 & 2. These DFM-PEG derivatives were purified using ion-exchange chromatography followed by gel filtration as described in Example 2. SDS-PAGE analysis revealed that conjugation to PEG was successful in all cases. (Figure 6). BIAcore analysis was also carried out to determine antigen binding affinity. Results shown in Table 4 demonstrate that these PEG derivatives can be attached with little loss in antigen binding affinity.

25

Table 4 BIAcore analysis of DFM-PEG derivatives

Sample	k _{ass}	k _{diss}	K _d (M)
DFM	1.81×10^7	2.29×10^{-3}	1.27×10^{-10}
DFM-PEG 5kDa (SCM linkage)	1.89×10^7	2.58×10^{-3}	1.37×10^{-10}
DFM-PEG 20kDa (SPA linkage)	9.80×10^6	2.37×10^{-3}	2.42×10^{-10}
DFM-PEG 10kDa (5kDa x 2, NHS linkage)	1.56×10^7	2.14×10^{-3}	1.38×10^{-10}
DFM-PEG2 20kDa (10kDa x 2, NHS linkage)	1.11×10^7	2.20×10^{-3}	1.99×10^{-10}

- 5 These DFM-PEG conjugates were then examined in a pharmacokinetic study in rats using the method described in Example 1. Results (Figure 7) demonstrate significantly slower blood clearance, (longer in vivo half-life) for the DFM-PEG conjugates compared to unmodified DFM.

10 Legends to Figures

Figure 1 SDS-PAGE analysis under non-reducing (lanes 1-5) and reducing (lanes 6-9) conditions.

Figure 2 Pharmacokinetics of ¹²⁵I-labelled anti-PDGFR DFM-PEG (40kDa, site-specific) compared to DFM and IgG in rats.

Figure 3 Inhibition of SK-5 proliferation response to 10ng/ml PDGF BB by anti-PDGFR DFM-PEG (40kDa random), DFM-PEG (40kDa site-specific), DFM-PEG (10kDa SS linker, site-specific), DFM and IgG.

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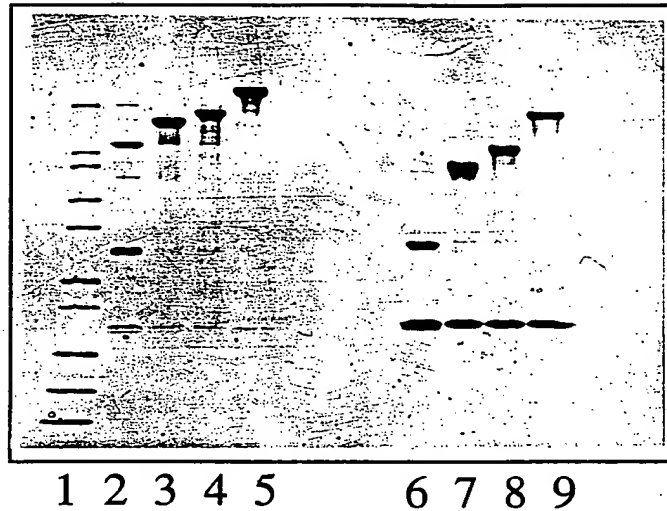
Figure 4 Inhibition of SK-5 proliferation response to 10ng/ml or 20ng/ml PDGF BB by 1 microgram/ml of anti-PDGFR DFM-PEG (40kDa random), DFM-PEG (40kDa site-specific), DFM-PEG (10kDa SS linker, site-specific), DFM and IgG.

25

Figure 5 Pharmacokinetics of ^{125}I -labelled anti-PDGF β R DFM-PEG (10kDa, SS linker) and DFM-PEG (20kDa, SS linker) compared to DFM in rats.

- 5 **Figure 6** SDS-PAGE analysis under non-reducing (lanes 1-7) and reducing (lanes 8-13) conditions.

Figure 7 Pharmacokinetics of ^{125}I -labelled anti-PDGF β R DFM-PEG (site-specific) of different types compared to unmodified DFM in rats.



Non-Reducing

1. Molecular weight marker proteins
2. Fab' and DFM mix
3. Purified DFM-PEG (10kDa SS linker)
4. Purified DFM-PEG (20kDa SS linker)
5. Purified DFM-PEG (40kDa)

Reducing

6. Fab' and DFM mix
7. Purified DFM-PEG (10kDa SS linker)
8. Purified DFM-PEG (20kDa SS linker)
9. Purified DFM-PEG (40kDa)

FIGURE 1

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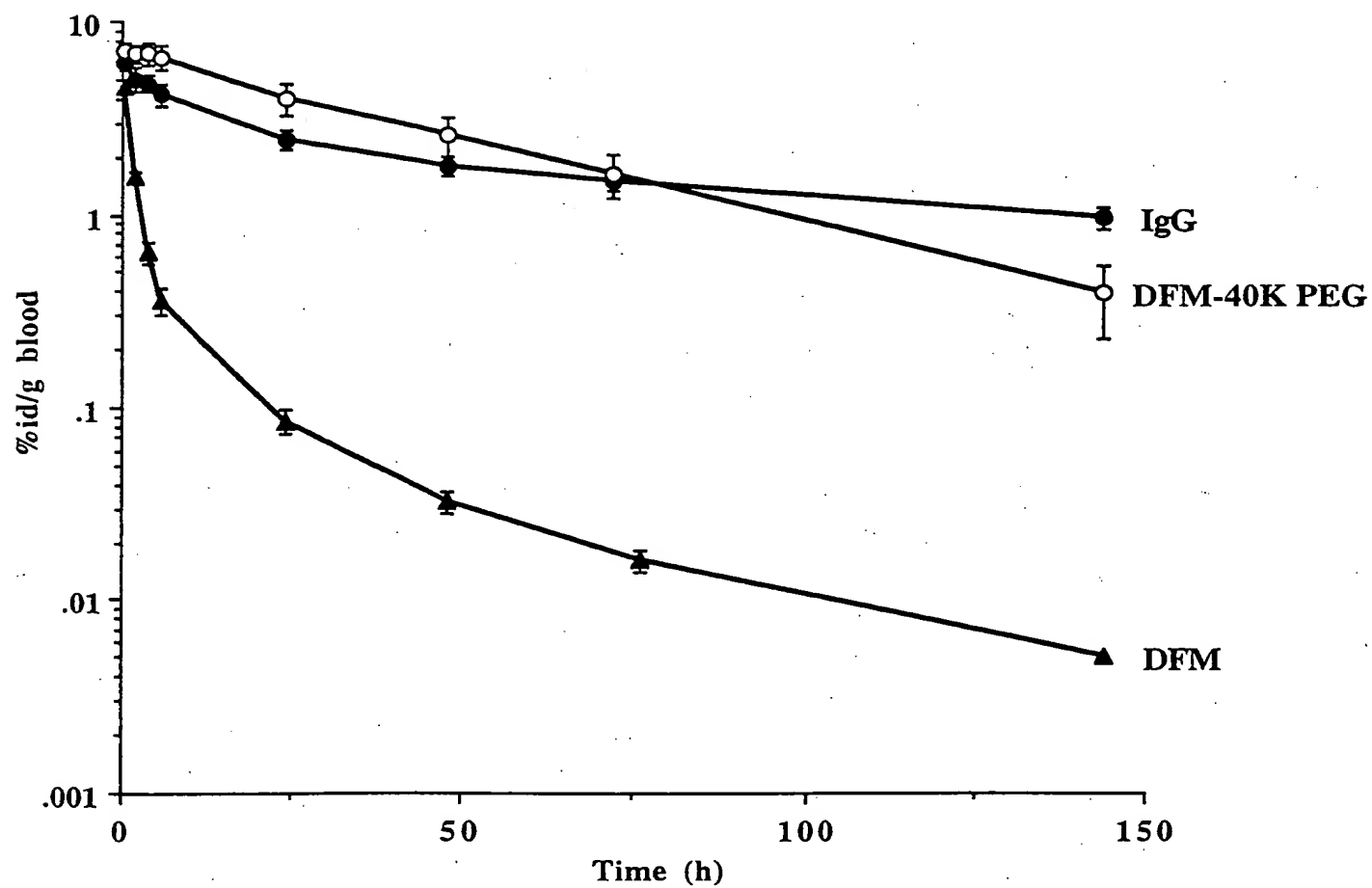
ECE29 &30 & 31 : Pharmacokinetics of 125-I anti-PDGF-R in rats

FIGURE 2

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Inhibition of SK-5 Proliferation response to 10 ng/ml PDGF BB by g162 antibody variants

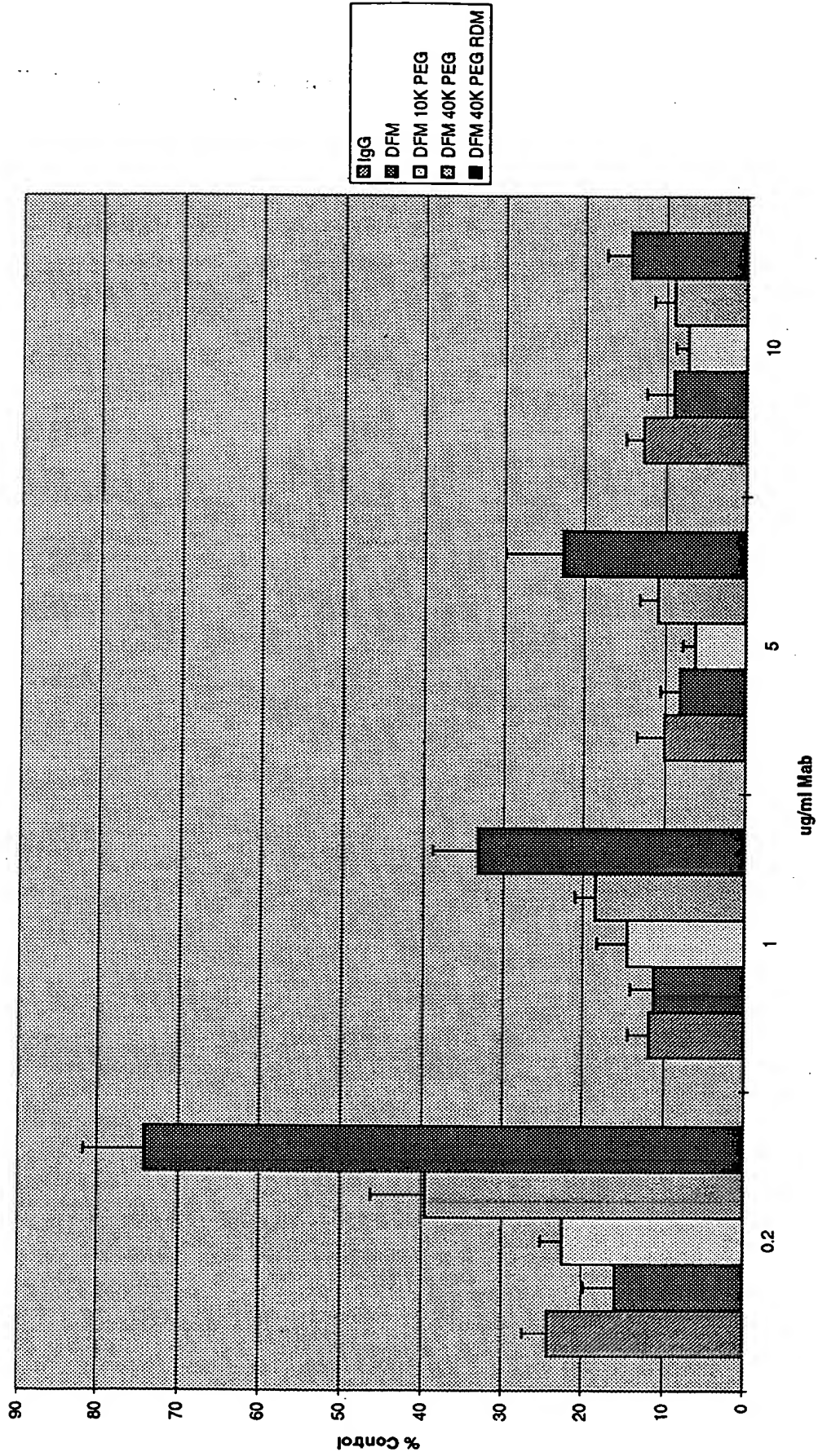


FIGURE 3

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Inhibition of SK-5 Proliferation response to PDGF BB by 1 ug/ml of g162 antibody variants

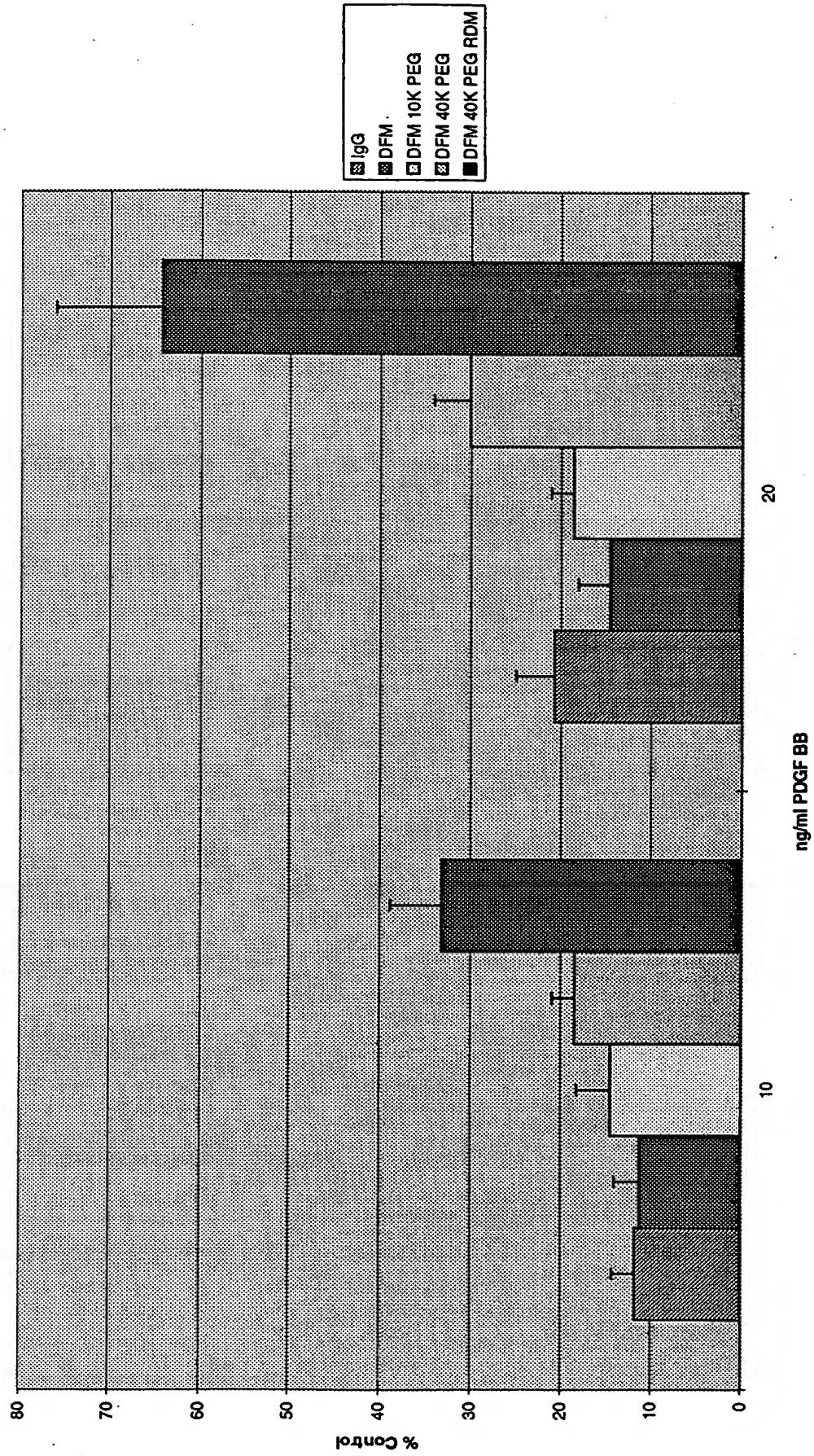


FIGURE 4

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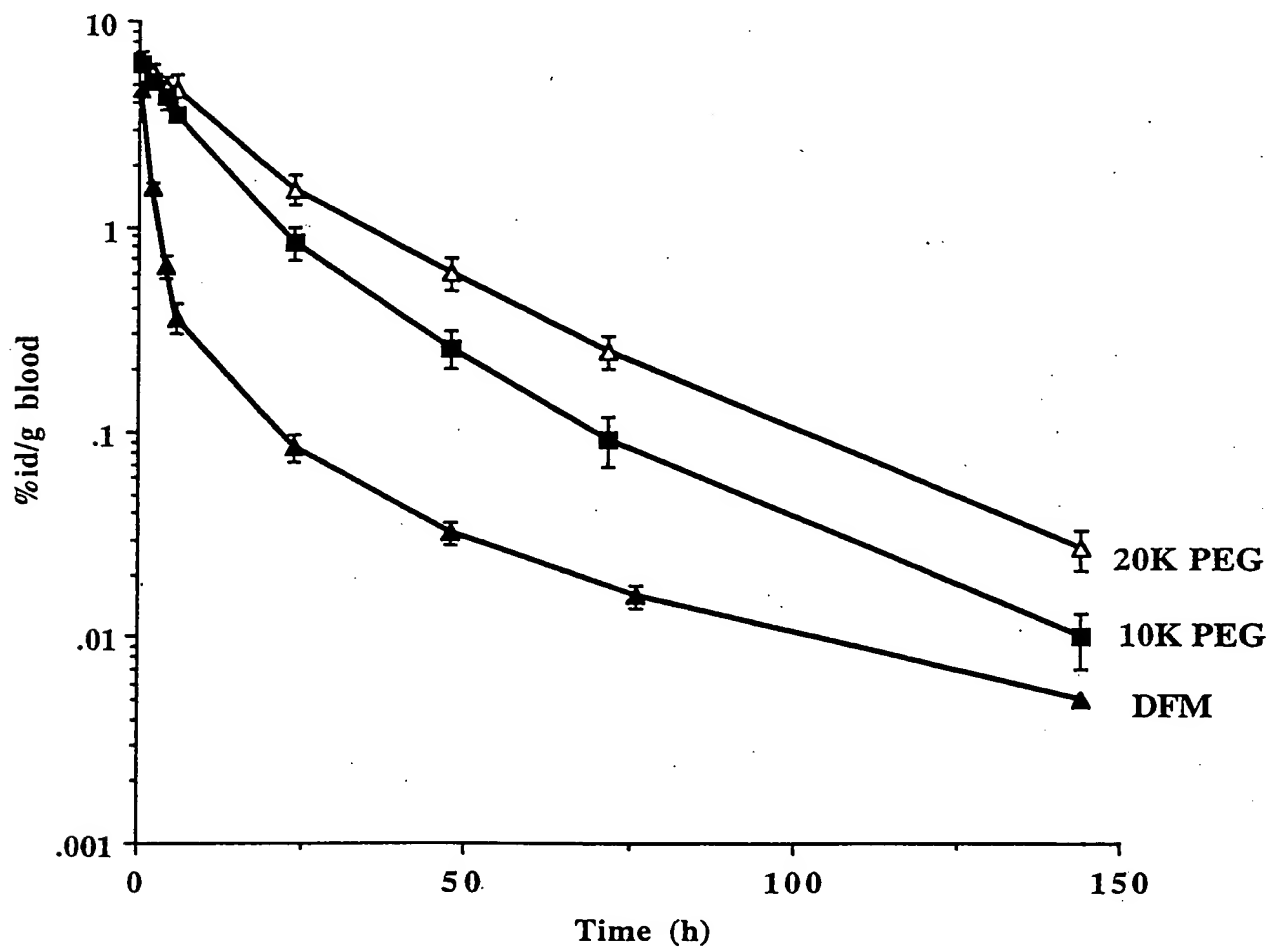
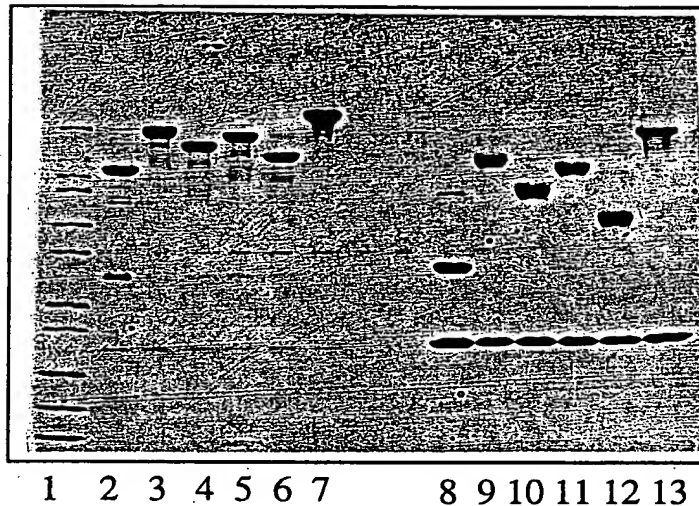
ECE29 & 31 : Pharmacokinetics of 125 -I anti-PDGF-R DFM in rats

FIGURE 5

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**Non-Reducing**

1. Molecular weight marker proteins
2. Purified DFM
3. Purified DFM-PEG (20kDa NHS linker)
4. Purified DFM-PEG (10kDa NHS linker)
5. Purified DFM-PEG (20kDa SPA linker)
6. Purified DFM-PEG (5kDa SCM linker)
7. Purified DFM-PEG (40kDa NHS linker)

Reducing

8. Purified DFM
9. Purified DFM-PEG (20kDa NHS linker)
10. Purified DFM-PEG (10kDa NHS linker)
11. Purified DFM-PEG (20kDa SPA linker)
12. Purified DFM-PEG (5kDa SCM linker)
13. Purified DFM-PEG (40kDa NHS linker)

FIGURE 6



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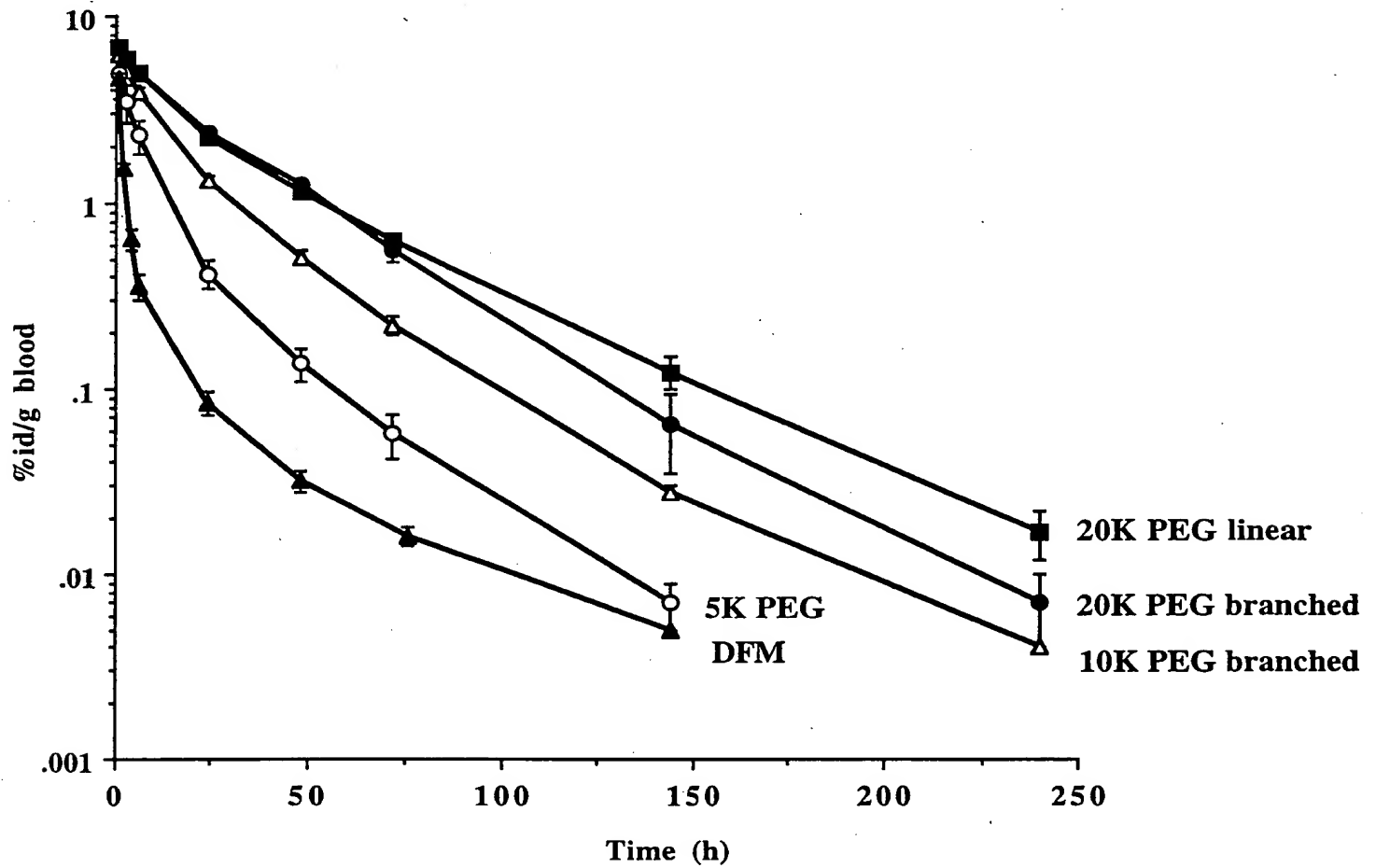
ECE29 & 32 : Pharmacokinetics of 125 I anti-PDGF-R DFM in rats

FIGURE 7

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